Reference: *Biol. Bull.* 189: 148–158. (October/November, 1995)

# Morphology and Physiology of the Thoracic and Abdominal Stretch Receptors of the Isopod Crustacean *Ligia exotica*

AKIYOSHI NIIDA, YOSHIKO TAKATSUKI*, AND TSUNEO YAMAGUCHI

*Department of Biology, Faculty of Science, Okayama University, Tsushima, Okayama 700, Japan*

**Abstract.** In the terrestrial isopod *Ligia exotica*, paired stretch receptors, each comprising a separate rapidly and slowly adapting receptor cell, were found in the third to eighth thoracic segments and first five abdominal segments. The dendritic endings of the two sensory cells in each receptor terminate on a common receptor muscle; the cross-striation of this fiber is homogeneous throughout the segments. But the dendritic endings of the receptor cells differ: the rapidly adapting cell has a club-shaped ending restricted to the middle of the receptor muscle, whereas the slowly adapting receptor cell has a bifurcating ending that extends along the entire length of the muscle. Stretch applied to the receptor muscle evokes characteristically different responses in the two sensory cells. The slowly adapting receptor cell has a lower firing threshold and fires continuously for the duration of the stretch, while the rapidly adapting receptor cell has a higher threshold and fires a brief burst at the beginning of the stimulus. However, application of an intense stimulus will evoke continuous firing of the rapidly adapting receptor, which then changes to intermittent bursts. The adaptive significance of such a response is not known, nor is it likely to occur in nature. However, this unusual response is intrinsic to the rapidly adapting cell, as it can be evoked by current injection. In the second thoracic segment, instead of rapidly and slowly adapting cells, we found a single slowly adapting cell with a long robust dendrite attached to the extensor muscle.

## Introduction

Phylogenetically, *Ligia* and the pill bug *Armadillidum vulgare* belong to the same suborder (Oniscoidea) of Isopoda. Both show a similar segmental pattern: a mobile thorax occupying a large part of the body and a reduced abdomen. The two animals also show distinct segmental movements of the body. The pill bug sluggishly rolls up in a spherical shape in response to noxious stimuli to its body or to the removal of its substratum. *Ligia*, in contrast, cannot roll up in this manner in response to such stimuli; rather, as in its swimming behavior, it shows the rapid upward and downward movements of the thoracico-abdominal segments. Niida *et al.* (1990) studied the stretch receptors that might correlate with the pill bug's sluggish conglobating behavior, and demonstrated that all the stretch receptors throughout thoracic and abdominal segments were of the slowly adapting type. Alexander (1971) recorded rapidly adapting discharges from the thoracic stretch receptors of *Ligia oceanica;* but slowly adapting stretch receptors, such as those in the abdomen of the crayfish *Procambarus clarkii* (Wiersma *et al.*, 1953), have not been reported in *Ligia*.

The existence of slowly adapting stretch receptors in *Ligia* is strongly suggested by the behavior described above, which surely requires postural controls. In addition, two types of stretch receptors—slowly and rapidly adapting—occur commonly in the abdomens of the decapod (Wiersma *et al.*, 1953) and stomatopod (Pilgrim, 1964). Even the N-cells, which are located in the most anterior segment of the thorax and have been considered as remnants of retrograde stretch receptors in the abdomen (Wiersma and Pilgrim, 1961), show slowly adapting impulse discharges in response to imposed stimuli. We thus assume that the slowly adapting stretch receptor should also predominate in *Ligia*.

The goal of this research is to understand the functional roles of the thoracic stretch receptor, especially anteriorly located ones, which would be closely related to segmental

Received 26 July 1994; accepted 26 July 1995.

* Present address: Department of Oral Science, Kyushu Dental College, Manazuru 2-61-1, Kokura, Kita-Kyushu 803, Japan.

movements. The present study thus characterized the stretch receptors of *Ligia exotica* both morphologically and physiologically. Some of the results presented here were reported in an earlier abstract (Takatsuki *et al.*, 1992).

## Materials and Methods

### *Animals*

Specimens of *Ligia exotica*, 30–35 mm in total length, were collected at the coast of the Seto Inland Sea near Ushimado Marine Laboratory, Faculty of Science, Okayama University, Japan. They were kept under a photoperiodic regime of 12 h light:12 h dark at 20°C. Both males and females were used in the experiments.

### *Identification of stretch receptors*

Conventional vital staining with methylene blue was used, as well as axonal filling with nickel chloride. In the latter staining technique, the cut distal stump of the dorsal nerve of the third nerve root in the thoracic ganglion was introduced into a glass capillary filled with 0.2 *M* $NiCl_2$. The preparation was stored at 4°C for 12–24 h to allow diffusion of the $NiCl_2$, which was precipitated by the addition of rubeanic acid. Stretch receptors identified by both staining methods were isolated and mounted in gelatin on glass slides.

### *Preparation for recording*

The responses of the stretch receptors to imposed stimuli were recorded *in situ* and *in vitro*. The following three types of preparations were used.

1. A semi-intact preparation was used when flexion was imposed *in situ*. After animals were anesthetized in cold seawater and decapitated, the legs and the 6th abdominal segment were cut off. The viscera were then dissected away from the cut end of the 6th abdominal segment, and the nerve cord was left intact. Such preparations were immediately flushed with seawater to prevent the deterioration of stretch receptors and nervous tissue by endogenous digestive enzymes.
2. A consecutive tergite preparation was used for imposed stretch experiments. The semi-intact preparation described above was cut with scissors along the midline of the sternite so that the trunk was bisected into two stripes of hemisegments from which the nerve cord was removed. These hemisegment preparations were then further cut into pieces of two consecutive tergites each.
3. A preparation of isolated stretch receptor was used for *in vitro* experiments. The dorsal nerve containing the axons of the stretch receptors was cut at its proximal end. The stretch receptor was then isolated by cutting the receptor muscle near its insertion.

Each of these three preparations, when complete, was then transferred to an experimental chamber filled with seawater. Most experiments were carried out in seawater cooled to 15–18°C. But at times a physiological saline for *Ligia*, prepared by Yamagishi (1985) based on the composition of *Ligia* serum (Parrey, 1953), was also used. We found no remarkable difference in impulse discharges for at least 5 h between seawater and physiological saline.

### *Stimulation and recording*

*Flexion experiments.* For extracellular recording from the stretch receptors in the 7th thoracic segment, all the anterior tergites up to the 6th thoracic segment were fixed ventral side up on a silver plate with instantaneous adhesive, while the free movable tergite of the 6th abdominal segment was pierced with a hook-shaped needle connected to the vertically moving central pin of a vibrator device (Fig. 1A). The vibrator device, with a frequency response from DC to 200 Hz, was driven by applying a ramp-and-hold pulse by which flexion size of the abdomen to the horizontal was varied from 0° to 60°. The flexion-induced responses were recorded from the dorsal nerve of the 3rd

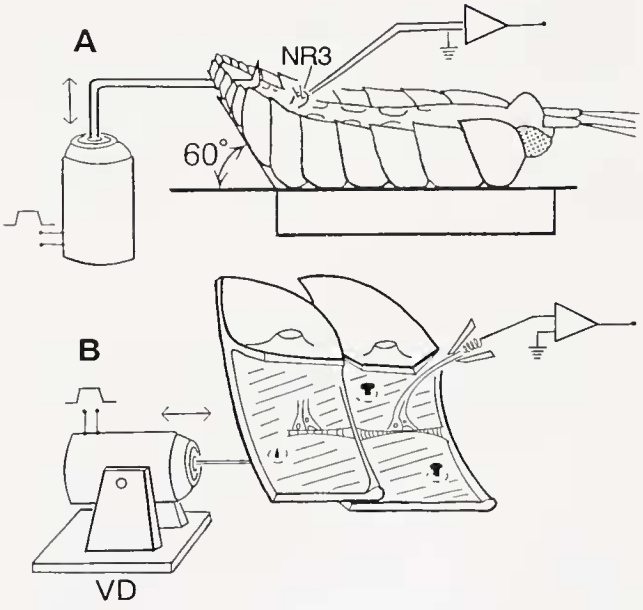


**Figure 1.** Experimental setup for recording responses from stretch receptors. (A) *In vivo* preparation. A flexion stimulus was delivered by a vibration device with an L-shaped arm that moves upward. In the experiments reported here, the flexion was produced by flexing the abdomen to 60° to the horizontal. Resultant responses were recorded with a tungsten hook electrode attached to the 3rd nerve root. (B) *In situ* preparation. One end of a pair of the bisected tergites was fixed with insect pins, and the other was connected to a vibration device that delivered the stretch stimulus. Stretch-induced activities of the receptor cells were recorded through a suction electrode attached to the distal cut end of the dorsal nerve of NR3. NR3, 3rd nerve root; VD, vibration device.

nerve root of the 6th thoracic ganglion with a tungsten hook electrode insulated by addition of mineral oil.

*Imposed stretch experiments.* The tergite just anterior to the segment containing the stretch receptors to be studied was fixed with an insect pin ventral side up. The free end posterior to the segment to be studied was connected to the horizontally moving pin of the vibrator device through the hook-shaped needle in the same manner as described above (Fig. 1B). A controlled stretch stimulus was thus delivered to the receptor muscle in the relevant segment, and the resulting responses were obtained from the dorsal nerve of the 3rd nerve root; the dorsal nerve was cut distally and introduced into a suction electrode made of a glass capillary.

*In vitro experiments.* Intracellular recordings were made for two purposes. The first was to determine which of the two receptor cells was responsible for a given response: *i.e.,* the slowly or rapidly adapting response to stretch stimuli. Each of the receptor cells was impaled with a glass microelectrode filled with 3 *M* KCl. Subsequently, imposed stimuli were delivered; each end of the receptor muscle of an isolated stretch receptor was gripped with a clamp mounted on a micromanipulator with which the receptor muscle was manually stretched. The second purpose was to analyze the characteristics of the intermittent discharges (described later) specific to the rapidly adapting stretch receptors of *L. exotica.* A bridge circuit was used in the analysis of the intermittent bursts, so current was injected into the receptor cell and the concomitant responses were recorded through a single microelectrode that was filled with 3 *M* KCl and had an impedance of 20–30 MΩ.

## Results

As described below, segmental stretch receptors occur bilaterally in the thoracic and the abdominal segments of *L. exotica.* For simplicity, the results will be described from one side only, and we refer to the thoracic and the abdominal stretch receptors as TSR and ASR, respectively. This study demonstrated the existence of slowly and rapidly adapting stretch receptors differing both physiologically and morphologically. A few animals did not respond to the stretch stimuli, where current injection into the stretch receptor cells produced a response comparable to that obtained by stretching. In *Ligia,* therefore, mechanical transduction might be greatly influenced by the mechanical deformation that occurs during the dissection of stretch receptors.

### *Spatial organization of stretch receptors*

The segmental trunk of *L. exotica* is composed of eight thoracic and six abdominal segments; because the first thoracic segment is fused with the head, the main part of the thorax forms seven segments, *i.e.,* the 2nd to 8th segments. Figure 2A shows the spatial organization of stretch receptors in the thoracic and abdominal segments, where each stretch receptor, except for TSR-1 (Fig. 2A), comprises a set of paired receptor cells and a single specialized receptor muscle. The TSR-1 possesses no specialized receptor muscle; instead, the musculature associated with the TSR-1 is an ordinary dorsal extensor muscle. The dendrite of the TSR-1, in its course, is partially attached to the articular membrane of the anterior ridge of the 3rd thoracic segment and runs toward its insertion in the anterior edge of the extensor muscle of the 2nd thoracic segment.

The characteristic organization of stretch receptors appears in TSR-2, which is located between the 3rd and 4th thoracic segments. A long receptor muscle (*ca.* 5 mm in 3.5 cm body length) has its posterior insertion on the anterior ridge of the 5th segment and runs through the 4th segment to the articular membrane of the anterior ridge of the 3rd segment. A pair of functionally differentiated receptor cells terminates on this receptor muscle within the 4th thoracic segment.

In the 5th thoracic segment, the anterior and posterior insertions of the receptor muscle lie on the individual anterior ridge of the 5th and 6th thoracic segments (Fig. 2A). This arrangement of the anterior and posterior insertions also occurs in the receptor muscle of the 6th thoracic segment. The arrangement in the most posterior of the thoracic segments is different again: anterior insertions of the receptor muscles of the 7th and 8th thoracic segments are in the connective tissue of each leg muscle of the 7th and 8th thoracic segments, whereas their posterior insertions occurred on the anterior ridges of the 8th thoracic and 1st abdominal segments, respectively (Fig. 2A).

TSR-1, as can be seen in Figure 2A, lies medially in association with the extensor muscle, but TSR-2 and subsequent stretch receptors lie somewhat dorsolaterally, and much more laterally than abdominal stretch receptors in the crayfish. The thoracic receptor muscles are shorter in successively more posterior segments, whereas abdominal receptor muscles become longer posteriorly (Fig. 2A).

### *Axonal pathway of stretch receptors*

The 3rd nerve root of each thoracic ganglion branches complexly: the dorsal nerve in this 3rd root provides a common pathway both for the central projection of the axons of stretch receptor cells and for efferents to the extensor muscle (Fig. 3A). A pair of the axons of the thoracic stretch receptor cells (rapidly and slowly adapting cells) bifurcate at the 3rd nerve root and course in two directions in pairs; one runs towards the subesophageal ganglion and the other towards the 6th abdominal ganglion (unpub. obs.). In the abdomen, the axons of the stretch receptor

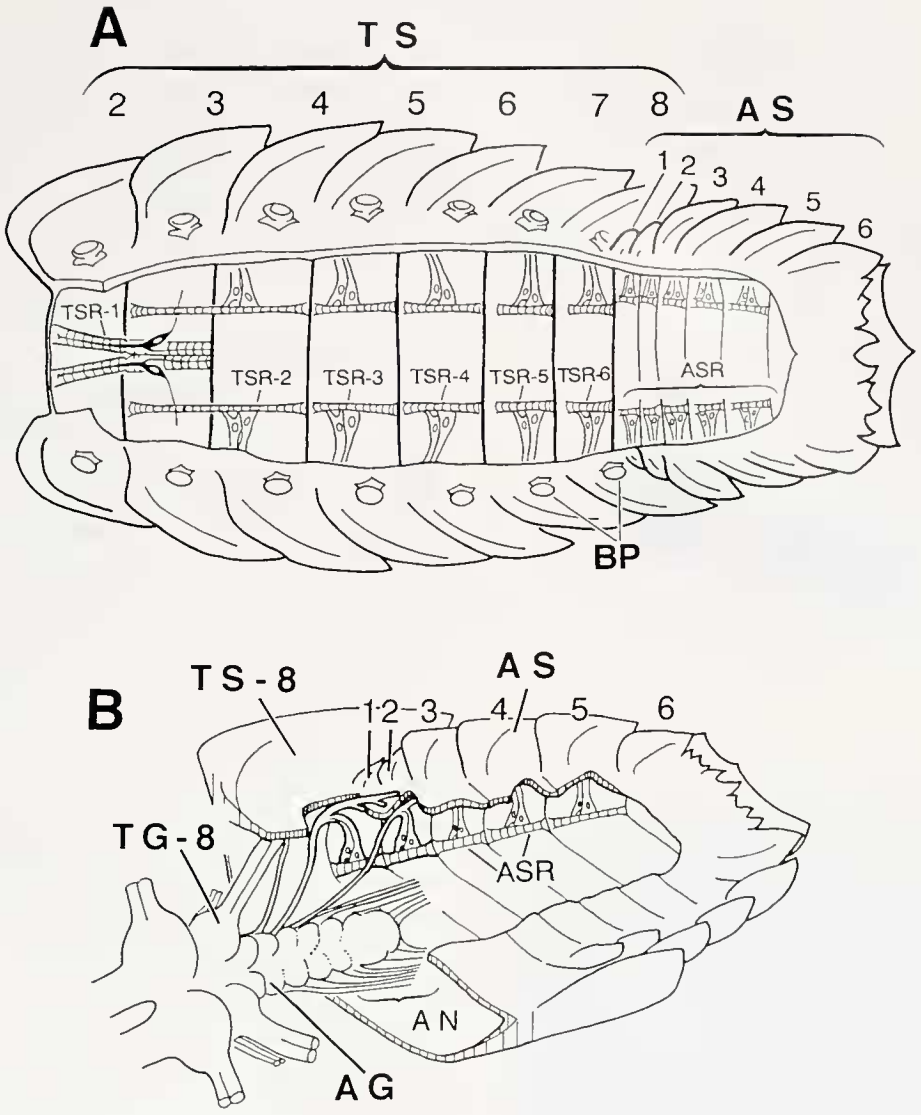


**Figure 2.** Organization of thoracic and abdominal stretch receptors (A) and the central connection of abdominal stretch receptors (B). (A) and (B) are viewed from the ventral side, and the viscera were removed as well as all muscles, except for those with which the stretch receptors are associated. In (A), head and legs are removed, and the first thoracic segment is not depicted because it is fused with the head. AG, abdominal ganglion; AN, abdominal nerve; AS, abdominal segment; ASR, abdominal stretch receptor; BP, basal propodite; TG-8, 8th thoracic ganglion; TS-8, 8th thoracic segment; TSR, thoracic stretch receptor. Numerals after TSR and ASR indicate position in the sequence of the segmental stretch receptors.

cells run through the abdominal nerve (Fig. 2B) and enter the several fused abdominal ganglia.

*Morphological characteristics*

*Thoracic stretch receptors.* TSR-1 has an extremely long dendritic process extending from a bipolar receptor cell located in the ventral surface of the medial extensor muscle of the 3rd thoracic segment (Fig. 2A). The dendritic process is quite stout in the anterior ridge of the 3rd thoracic segment, but as it extends forward, it gradually thins, running in close contact with the extensor muscle. The dendrite is attached to the muscle at its anterior extremity in the 2nd thoracic segment. Thus, although the length of the dendritic process depends on the total body length, in animals 3 to 4 cm long, it measures 2 to 3 mm from the receptor cell soma. Running posteriorly, a thin strand originates from the initial part of the stout dendrite, but its insertion could not be traced precisely.

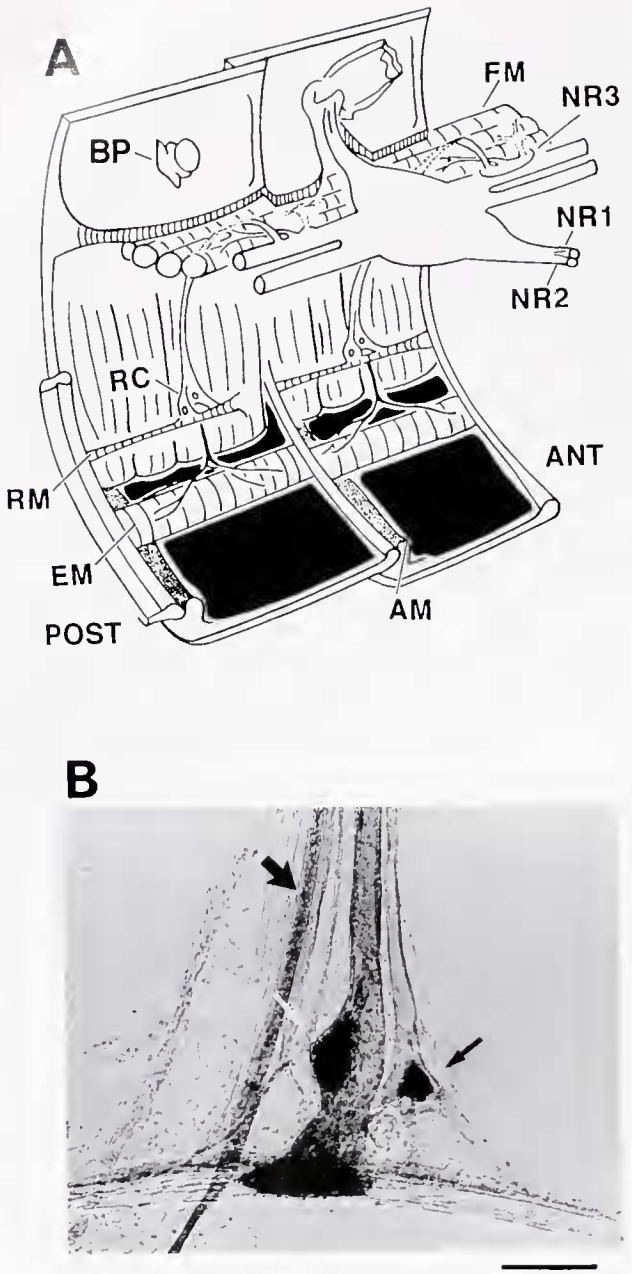


**Figure 3.** (A) Spatial arrangement of stretch receptors in the 6th and 7th thoracic segments and the central connections of stretch receptors. The figure, viewed from the ventral side, shows hemisegments with part of the lateral side of the tergites not depicted. NR1 and 2 run towards the pereopod, whereas the dorsal nerve of NR3 provides a common pathway for the central projection of the axon of the stretch receptors and for the efferent fiber to the extensor muscle (EM). Note that the anterior insertion of receptor muscle of the 7th thoracic segment occurs in the leg muscle of the same thoracic segment. (B) Photomicrograph of a thoracic stretch receptor (TSR-3) stained by axonal filling with nickel chloride. Thick arrowhead indicates an efferent to the extensor muscle. Thin white and black arrowheads indicate C-type (rapidly adapting) and B-type (slowly adapting) stretch receptors, respectively. Scale bar, 200 μm. ANT, anterior; AM, articular membrane; BP, basal protopodite; EM, extensor muscle; FM, flexor muscle; NR1, 1st nerve root; NR2, 2nd nerve root; RC, receptor cells; RM, receptor muscle; POST, posterior.

With the exception of TSR-1, the receptor cells of the stretch receptors in both thoracic and abdominal segments were classified morphologically into two different types on the basis of their dendrites: club-shaped cells (C-type) and bifurcating cells (B-type) (Fig. 3B). The characteristics of these cell types emerge from schematic illustrations of the stretch receptors in the 2nd to 8th thoracic segments (Fig. 4). Each C-type cell shows a stout dendrite attached to the central part of the receptor muscle. In contrast, the branching dendrites of B-type cells run in both directions along the total length of the receptor muscles. The dendritic processes of both C- and B-type cells are much longer in TSR-2 than those of the stretch receptors in other thoracic segments. Another characteristic of the stretch receptors is the homogeneous striation of the receptor muscles throughout thoracic and abdominal segments. Although systematic measurements were not made, the sarcomere length of the receptor muscle in the 7th thoracic segment was 3.6 ± 0.18 (mean ± SD) μm. Of course, this muscle—in every segment—is shared by slowly and rapidly adapting stretch receptor cells. In the crayfish, however, the sarcomeres are short (3.3 μm) in the receptor muscle of the rapidly adapting stretch receptor, and long (6.5 μm) in that of the slowly adapting stretch receptor (Komuro, 1981).

*Abdominal stretch receptors.* The morphology of the abdominal stretch receptors (Fig. 5) is similar in general to that of the thoracic receptors: *i.e.*, there are C-type and B-type receptor cells, and they are attached to the single receptor muscles with homogeneous striations. But the B-type receptor cells of the 2nd and 3rd abdominal segments show morphological variations in the manner of the bifurcation of their dendrites. Generally, the dendrites of the B-type receptor cells in the 2nd and 3rd abdominal segments bifurcated in close contact with the receptor muscle, as in ASR-1 (Fig. 5), but some receptor cells show a dendritic branching pattern; *e.g.*, in ASR-4 the dendrite branches distally to the receptor muscle. Another difference from the TSR is that the dorsal extensor muscle closely parallels the abdominal receptor muscle. This anatomical arrangement closely resembles that of the crayfish, *Procambarus clarkii* (Wiersma *et al.*, 1953).

## In situ *response of stretch receptors to imposed flexion*

Figure 6 shows a representative *in situ* recording from TSR-5 of the 7th thoracic segment in response to abdominal flexion in the ventral direction (upward imposed flexion). The flexion was imposed with a vibrator device driven by a ramp-and-hold pulse of 0.05 Hz; the animal was ventral side up, and the abdominal flexion was 60° from the horizontal axis (Fig. 1A). The evoked responses showed slowly and rapidly adapting impulse discharges or phasic and tonic responses, as shown in

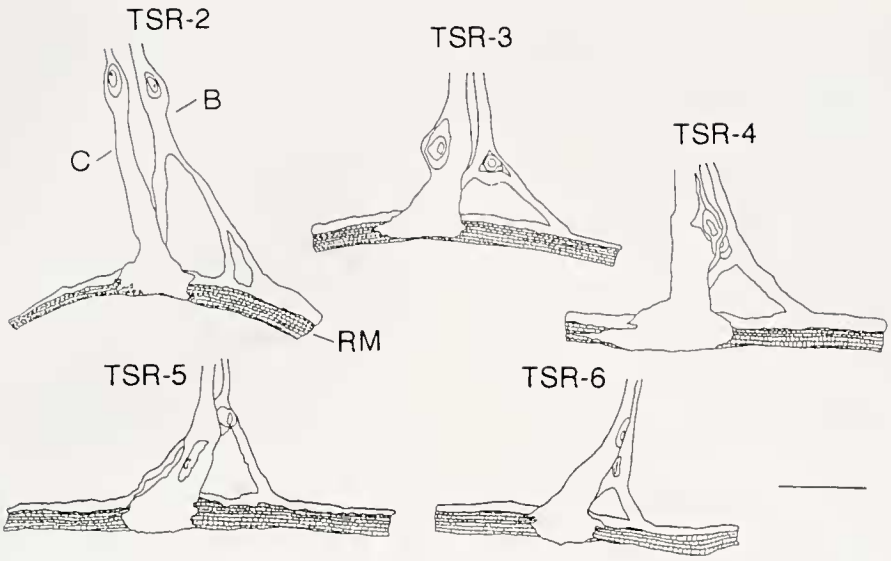


**Figure 4.** Schematic drawings of thoracic stretch receptors. The drawings in this and the next figures were based on specimens stained with methylene blue. B, B-type cell; C, C-type cell. Note that the cross-striation within every receptor muscle, from TSR-2 to TSR-6, is homogeneous. Scale bar, 100 $\mu$m.

the inset of Figure 6. The adaptive time courses of impulse discharges from rapidly and slowly adapting stretch receptors are also shown in Figure 6a and b. The phasic response has longer latency due to the slow rise of the stimulus delivered at 0.05 Hz; the phasic quality indicates dependence on the velocity; *i.e.*, the rate of displacement (angle/s) of the thoracic segment by the flexion stimulus (Fig. 7). Similar responses accompanied by tonic impulse discharges were recorded from the TSR-5 upon abdominal extension (downward imposed flexion) (data not shown). Extension produced much lower impulse frequencies than flexion, even when the degree of the applied stimulus was the same. In the presence of motor activities of the extensor neuron,

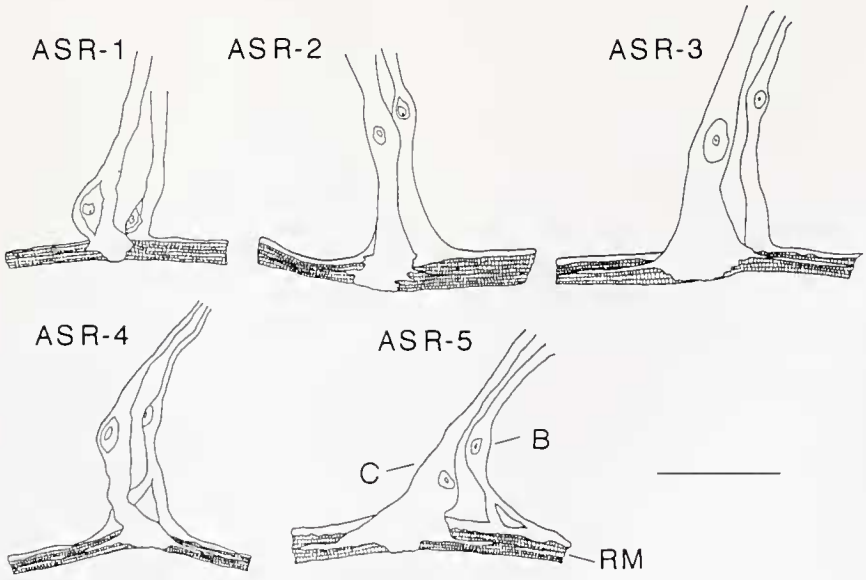


**Figure 5.** Schematic drawings of abdominal stretch receptors. Homogeneous cross-striation is similarly noted in the abdominal receptor muscles. B, B-type cell; C, C-type cell. Scale bar, 100 $\mu$m.

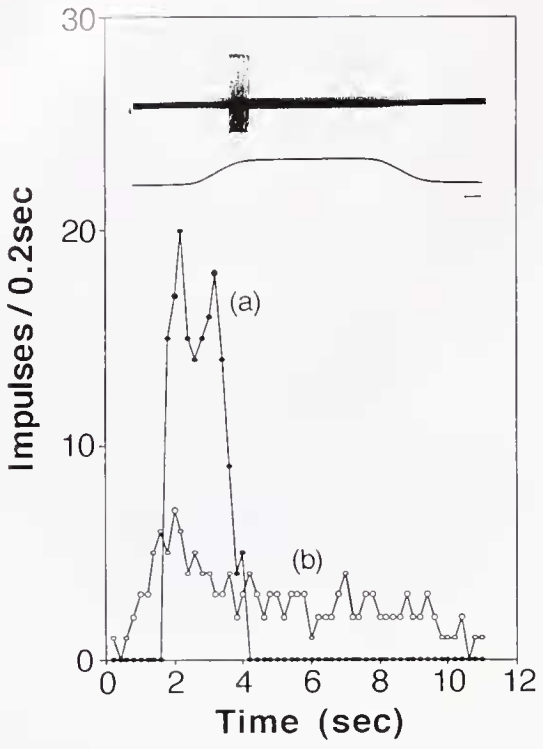


**Figure 6.** Response of TSR-5 (*in situ* preparation) to imposed flexion. (a) and (b): the time courses of impulse discharges in the rapidly and slowly adapting stretch receptors, respectively. Inset: responses from the two types of stretch receptors. Lower trace, flexion amplitude (60° from horizontal). Time scale, 1 s.

however, impulse frequencies of the TSR-5 were somewhat increased (data not shown).

*Response of thoracic stretch receptors to stretch stimuli*

Both slowly and rapidly adapting impulse discharges were evoked from each stretch receptor in the 3rd to 8th thoracic segments by an imposed stretch stimulus. In contrast, TSR-1 in the most anterior segment is a simple stretch receptor and its response is only slowly adapting (not shown). To represent activities of the stretch receptors with both slowly and rapidly adapting cells, the records from TSR-2 are shown in Figure 8, and two kinds of impulse discharges differing in their frequency and amplitude can be seen (Fig. 8A). One was derived from a slowly adapting receptor cell and showed a tonic impulse discharge that gradually adapted as long as the receptor muscle was stretched. In this particular receptor, the ongoing tonic impulse discharges appeared before the stretch stimulus because we extended the receptor muscle slightly while securing the thoracic segment with insect pins to the cork platform in the experimental chamber. In this experiment, therefore, we took the initial length of the receptor muscle with the slight extension as its apparent zero length.

When the receptor muscle was stretched in increments of 0.03 mm (Fig. 8B), a notable phasic response occurred at an increment of 0.25 mm from the relative zero length of the receptor muscle (Fig. 8B). This indicates that the cells showing a phasic response possess a higher threshold for a given length of stretch than cells showing a tonic response, and they might be more sensitive to transient segmental movement. On the other hand, tonic cells might serve as positional detectors: thus, when the receptor muscle was stretched in steps of 0.05 mm, up to 0.9 mm, a linear relationship was observed between impulse frequency and the length of stretch in the range of 0.45 to 0.9 mm (Fig. 8C). This relationship holds good only in the dynamic range of the stretch receptor; *i.e.*, the impulse discharge saturates when the stretch stimulus is much larger (see Fig. 10b).

Identification of particular response characteristics to either B-type or to C-type cells was demonstrated by intracellular recording. A microelectrode was used to penetrate either B-type or C-type cells that had been identified under a binocular microscope. When stretch stimuli or current injections were applied, a slowly adapting response was recorded from the B-type (not shown), and the rapidly adapting response was recorded from the C-type receptor cells (Fig. 9B).

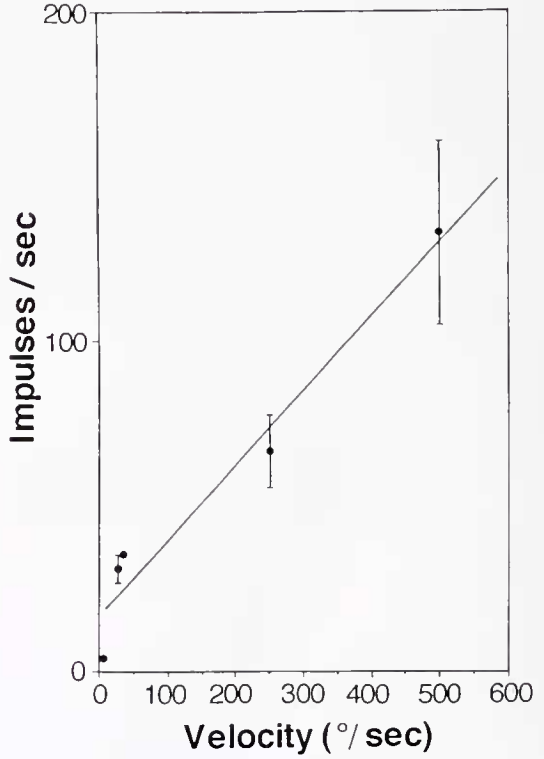


**Figure 7.** Effect of the velocity of imposed flexion on the response of stretch receptors. These data were obtained by varying the ramp slopes in the experiment shown in Figure 6. Points with vertical bars represent mean ± SD.

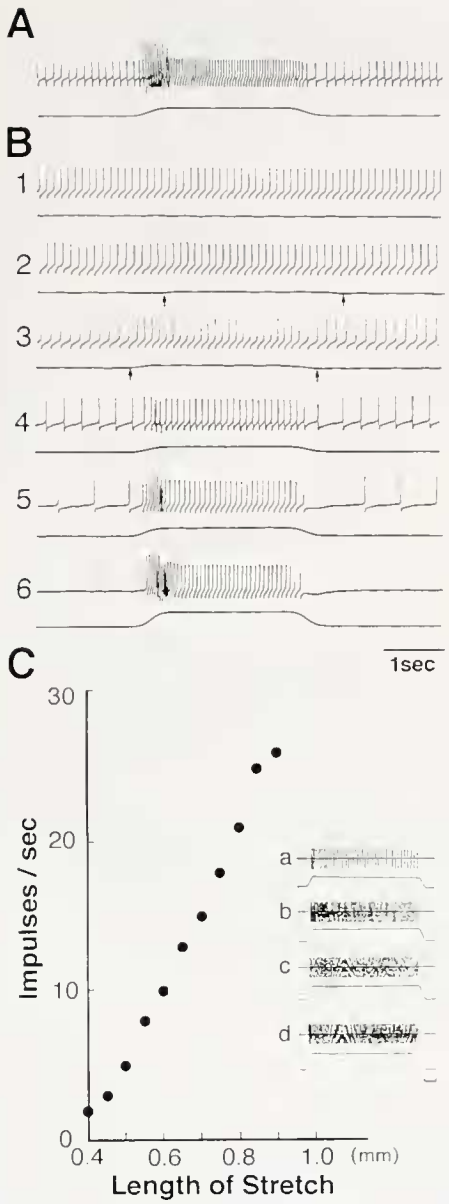


**Figure 8.** (A) Typical stretch-induced response of the *in situ* thoracic stretch receptor, TSR-2, showing both rapidly and slowly adapting responses. Stretch amplitude, 0.3 mm. (B) Responses of TSR-2 in the same specimen as in (A) to various lengths of stretching. In (A) and (B), the lower record in each pair represents the relative amplitude of the applied flexion. $B_1$, 0 mm; $B_2$, 0.03 mm; $B_3$, 0.06 mm; $B_4$, 0.09 mm; $B_5$, 0.12 mm; $B_6$, 0.25. Arrows indicates beginning and cessation of stretch stimulus. (C) Relationship between length of stretch and frequency of impulses. These data were taken from an *in situ* preparation of TSR-5 obtained from a specimen different from that in (A). Inset shows some of the responses and stimulus amplitude (lower traces) that were plotted. (a) to (d) correspond to stretch of 0.5, 0.6, 0.7, and 0.8 mm, respectively. Time scale (inset), 2 s. In (A), (B), and (C), recordings were made extracellularly through a suction electrode.

A closer examination of the phasic response led to unexpected results. When the receptor muscle was stretched beyond a certain length, the usual pattern of the phasic response changed to maintained discharge of intermittent bursts (Fig. 9A). In this case, the receptor (TSR-5) was stimulated with a 0.6-mm stretch. Within 8.4 s after the onset of stimulus, a stretch-induced response with the usual impulse discharge pattern of a rapidly adapting stretch receptor occurred. But by 8.6 s after the onset of the stimulus, intermittent bursting began and lasted for the duration of the stretch stimulation. In Figure 9A, two groups of impulse bursts appear at the rising phase of stimulation (arrowheads). This was caused by the unevenness of manually imposed stretch stimulus. Intermittent bursts equivalent to those evoked by stretch stimulus were also produced by intracellular injection of electrical current (Fig. 9B, 5nA in this case). The occurrence of this phenomenon is illustrated graphically in Figure 9C and D. No intermittent bursts occurred after a current injection of 3nA (Fig. 9C) and the evoked responses ceased in about 4 s. But at 8nA (bottom line in Fig. 9D), and about 18 s after current injection, intermittent bursts appeared and lasted for 40 s. The outcome of this experiment is shown in the inset of Figure 9D, with intermittent bursts still occurring in the penultimate 10 s of a 5-min stimulus.

However, the question remained: Could this phenomenon be produced by a much stronger imposed stimulus beyond the functional range of the receptor? This possibility might be excluded by the observation that the frequency of intermittent bursts increased linearly until 0.8 mm (Fig. 10a). Because we adopted the stretch stimulus of 0.6 mm, we could exclude the above possibility. This stretch amplitude was also within physiological range of the tonic response cell. The tonic response cell, which was simultaneously activated (since both type of receptor cells have a common single receptor muscle), responded with an increase of impulse discharges, even up to 1.0 mm (Fig. 10b), corresponding to a 30% increase in the total length of the receptor muscle.

### *Response of the abdominal stretch receptor*

As can be seen in Figure 11, slowly adapting and rapidly adapting responses also appeared in the abdominal stretch receptors (inset of Fig. 11). These responses are similar to those in the thorax, but the rapidly adapting receptor cells of the abdomen never showed the intermittent bursts observed in those of the thorax. As in the thorax, instead of the stretch, a certain amount of current injection could cause an equivalent response in the stretch receptors. Injection of 4nA (Fig. 11a), or even the much larger current of 8nA (Fig. 11b), induced no intermittent bursts in the abdomen. Although a current of 8nA was sufficient to evoke intermittent bursts in thoracic receptors, injection even beyond 8nA generated no intermittent bursts in the abdominal stretch receptors.

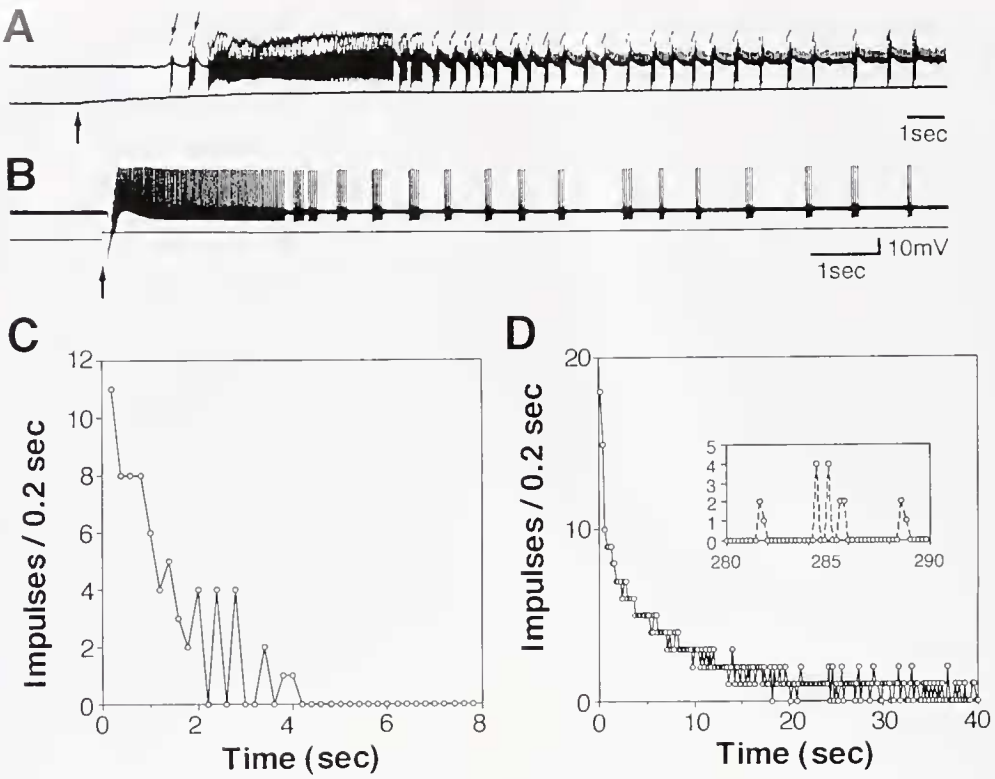


**Figure 9.** (A) Intermittent bursts in response to an imposed stretch, recorded extracellularly from the rapidly adapting receptor cell in an *in situ* preparation of TSR-5 to an imposed stretch. Stretch amplitude (lower trace), 0.6 mm. Thin arrows indicate impulse bursts caused by an uneven stretch stimulus imposed manually. The small impulse discharges indicate the activities of the slowly adapting stretch receptor. (B) Similar intermittent impulse discharges produced by current injection applied intracellularly; recordings were made intracellularly from a C-type cell. Thick arrows in (A) and (B) indicate the onset of stretch stimulus and current injection, respectively. (C) A current of 3 nA caused no intermittent bursts. (D) At 8 nA, intermittent bursts occurred about 20 s after beginning current injection; they were maintained throughout a 5-min stimulus (inset).

## Discussion

### *Intrinsic response property of the rapidly adapting stretch receptor*

The intermittent bursts of the rapidly adapting receptor cell that were observed during imposed stretch experiments were dependent on the extent of a stretch stimulus. As can be seen in Figure 10a, stretching within the range of 0.6 to 0.8 mm would not be unusual, because concomitant impulse discharges increased with the increment of stretch. This type of response is clearly specific to the thoracic rapidly adapting stretch receptor cells, because application of electrical current to the rapidly adapting stretch receptors of the abdomen did not alter their response pattern and evoked no intermittent bursts (a, b in Fig. 11). Such a stable response pattern as that in the abdomen of *L. exotica* occurs also in the rapidly adapting abdominal stretch receptors of crayfish (Nakajima and Onodera, 1969), which showed a phasic response with any intensity of applied electrical current.

On the other hand, segmental *in situ* flexion induced no intermittent bursts (Fig. 6). One reason for this inconsistency might be the absence or presence of inhibitory inputs from central neurons to the stretch receptor cells; specimens for stretch-imposed experiments are isolated from the central connection. An unequal stimulus amplitude between imposed flexion and stretch experiments might also account for the difference in response.

### *Segmental mobility and response type of stretch receptor*

Unlike the segments in large crustaceans such as crayfish, all isopod segments are mobile, suggesting that all of the stretch receptors should be equipped with a specialized receptor muscle on which the dendrite of the receptor cell terminates. This assumption is derived from a concept by Bush and Laverack (1982): in the Crustacea, evolution progresses with increasing sclerotization, and thoracic segments are consequently immobilized; anterior rapidly adapting stretch receptors are lost first, followed by slowly

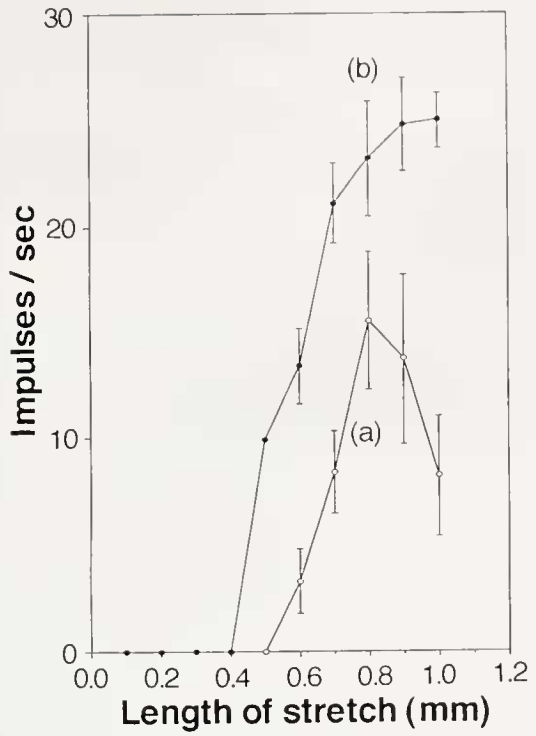


**Figure 10.** Frequency plots based on recording from two receptor cells (TSR-5) to various lengths of stretch. (a) and (b): rapidly and slowly adapting receptor cells, respectively. Paired slowly and rapidly adapting receptor cells were simultaneously activated, because they have a single common receptor muscle. Points with vertical bars represent mean ± SD.

adapting stretch receptors; these are finally replaced by N-cells, which have no specialized receptor muscles. The stretch receptor of the 2nd thoracic segment of *Ligia* has no specialized receptor muscles, a lack also reported in pill bugs (Niida *et al.*, 1990). This type of stretch receptor may be equivalent to the N-cells of large decapods (Alexandrowicz, 1952; Wiersma and Pilgrim, 1961) and of *Squilla mantis* (Crustacea, Stomatopoda) (Pilgrim, 1964). *S. mantis* has "free" thoracic segments that are mobile; thus, in contrast to the Decapoda, this species contains a complete set of stretch receptors, each with a specialized receptor muscle and a receptor cell, from the abdominal segment up to the 5th thoracic segment.

In *Squilla*, the N-cell, termed SR-$\alpha$ (Wiersma and Pilgrim, 1961), lies only in the 2nd thoracic segment. This segmental organization is the same as that in *Ligia*, but the responses of the stretch receptors of the 3rd thoracic segment differ in these two animals. The response in *Squilla* is only of the slowly adapting type, whereas the receptors in *Ligia* show both slowly and rapidly adapting responses. Therefore, the 3rd thoracic segment may be more mobile in *Ligia* than in *Squilla*. The appearance, within the Isopoda, of a segmentally arranged series of stretch receptors comprising sensory cells of two types thus further supports the hypothesis by Alexandrowicz (1967): the organization of the thoracic stretch receptors is closely related to the mobility of the thorax.

### *Comparison of the structure of receptor muscle in other Crustacea*

As already stated, the receptor muscle of *Ligia* is a single structure throughout each segment. In large Crustacea, such a single receptor muscle appears in the anterior thoracic segments; *e.g.*, *Astacus* has it in the 7th thoracic segment, *Homalus* in the 7th thoracic segment, and *Squilla* in the 3rd and 4th thoracic segments (for review, see Bush and Laverack, 1982). The more posterior thoracic segments and successive abdominal segments of each animal have two separate receptor muscles. In the pill bug (Niida *et al.*, 1990; Niida *et al.*, 1991), unlike *Ligia*, the receptor muscle that spans the 3rd and 4th thoracic segments separates completely, and from the 5th to 8th thoracic segments each pair of receptor muscle runs closely together—but Moser's observation (1976) is somewhat different from ours. In the abdomen, parallel receptor muscles connect tightly with each other in the anterior ridge of a targum and run toward the adjacent segment, separating into two muscle components. Thus the variation in the organization of the receptor muscle might be difficult to account for on the basis of evolutionary sequence alone in a limited number of animals; adaptive behaviors specific to the relevant animal should be also considered.

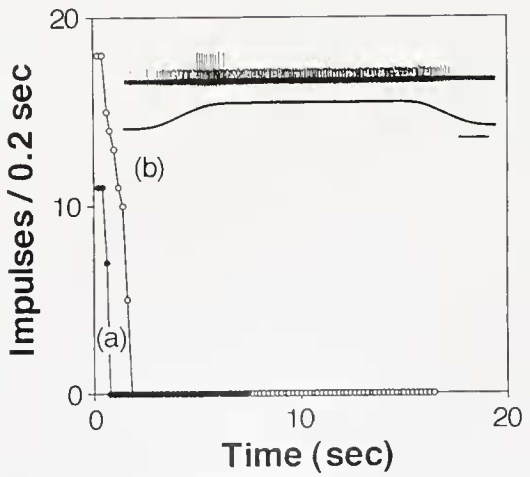


**Figure 11.** Inset: Extracellularly recorded responses from rapidly and slowly adapting abdominal stretch receptors (ASR-2). Lower trace, stretch amplitude (0.22 mm). Time scale, 2 s. Graph: Time courses of impulse discharges by the rapidly adapting receptor cell of ASR-2. Impulse discharges recorded intracellularly were evoked by intracellular current injection. (a), 4 nA; (b), 8 nA.

The sarcomere length, as one of the characteristics of the differentiated receptor muscle, may be noted; because in crayfish, a slowly adapting receptor cell and a rapidly adapting receptor cell have receptor muscles with a long sarcomere and a short one, respectively (Komuro, 1981). The same is the case with *Squilla* (Alexandrowicz, 1967). In contrast, in *Ligia*, microscopic observation of the cross-striation of the receptor muscles (not measured except in the 6th thoracic segment) did not reveal any difference in sarcomere length in each segment. Accordingly, the differentiated function of stretch receptors in *Ligia* should be attributable to the specific morphology of receptor cells (B-type, C-type cells) coupled with their response properties, rather than to sarcomere length.

When this receptor muscle is passively stretched, both of the receptor cells associated with it (B-type and C-type) should be synchronously stimulated, but the behavioral significance is difficult to evaluate.

### *Adaptive behavior and thoracic stretch receptor*

The slowly adapting stretch receptor of the crayfish has been regarded as a positional detector of abdominal flexion, whereas the rapidly adapting stretch receptor is believed to function when the fast muscular system is activated, such as during swimming and escape (Wiersma and Pilgrim, 1961). In *L. exotica*, the abdominal and posterior thoracic segments, as well as the uropod styles, flex in the dorsal and ventral directions. This segmental movement is related to swimming, but is also sequentially elicited by another key stimulus: When pereopods of *L. exotica* are dipped in a large quantity of water, the animal attempts to stand, elevating its body and beginning to raise and lower its styles to the substrate. This behavior might be coupled with the water-conducting system that has been extensively studied (for review, see Warburg, 1993; Hoese, 1984). In this system, water is taken up from water droplets by the capillary action of the pereopods and enters the marsupium; the extra water is released by the touch of the styles to the ground. In performing this behavior, the animals must obtain continuous, momentary information about the position of their styles with respect to the ground and about the velocity of flexion. In the anterior thoracic segments, although the functional roles of the rapidly and slowly adapting stretch receptors are unclear, both types of stretch receptors would be required for sophisticated segmental movements. For instance, when opening the breeding pouch, which occurs in the 2nd to posterior thoracic segments of females, the animal presses its anterior thoracic segments against the substrate and simultaneously lifts its posterior segment by supporting the abdominal segments with the styles.

## Acknowledgments

This work was supported by Ryoubi Teien Foundation and in part by a Grant in Aid from the Ministry of Education, Science and Culture of Japan to TY for scientific research.

## Literature Cited

**Alexander, C. G. 1971.** Observations on receptor mechanisms in *Ligia oceanica* (Linn.) *Comp. Biochem. Physiol.* **40A:** 339–347.

**Alexandrowicz, J. S. 1952.** Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palmurus vulgaris*. *Quart. J. Microsc. Sci.* **93:** 315–346.

**Alexandrowicz, J. S. 1956.** Receptor elements in the muscles of *Leander serratus*. *J. Mar. Biol. Ass. UK* **35:** 129–144.

**Alexandrowicz, J. S. 1967.** Receptor organs in thoracic and abdominal muscles of Crustacea. *Biol. Rev.* **42:** 288–326.

**Bush, B. M. H., and M. S. Laverack. 1982.** Mechanoreception. Pp. 399–468 in *The Biology of Crustacea*, Vol. 3, H. L. Atwood and D. C. Sandeman, eds. Academic Press, New York.

**Hoese, B. 1984.** The marsupium in terrestrial isopods. Pp. 65–76 in *Biology of Terrestrial Isopods*, S. L. Sutton and D. M. Holdich, eds. Oxford University Press, New York.

**Komuro, T. 1981.** Fine structural study of the abdominal muscle receptor organs of the crayfish (*Procambarus clarkii*). Fast and slow receptor muscles. *Tissue & Cell* **13:** 79–92.

**Moser, H. 1976.** Muscle receptor organs (MRO) in Isopoda (Crustacea)—histological observations. *Mikroskopie* **31:** 350–362.

**Nakajima, S., and K. Onodera. 1969.** Membrane properties of the mechanism of sensory adaptation. *J. Physiol.* **200:** 161–185.

**Niida, A., K. Sadakane, and T. Yamaguchi. 1990a.** Stretch receptor organs in the thorax of a terrestrial isopod (*Armadillidium vulgare*). *J. Exp. Biol.* **149:** 515–519.

**Niida, A., K. Sadakane, and T. Yamaguchi. 1991.** Abdominal stretch receptor organs of *Armadillidium vulgare* (Crustacea, Isopoda) *Zool. Sci.* **8:** 187–191.

**Parrey, G. 1953.** Osmotic and ionic regulation in the isopod crustacean *Ligia oceanica*. *J. Exp. Biol.* **30:** 567–574.

**Pilgrim, R. L. C. 1964.** Stretch receptor organs in *Squilla mantis* Latr. (Crustacea: Stomatopoda). *J. Exp. Biol.* **41:** 793–804.

**Takatsuki, Y., A. Niida, and T. Yamaguchi. 1992.** Stretch receptor organs in the thorax and abdomen of *Ligia exotica* (Crustacea, Isopoda). *Zool. Sci.* **9:** 1243.

**Warburg, M. R. 1993.** *Evolutionary Biology of Land Isopods*. Springer Verlag, Berlin.

**Wiersma, C. A. G., E. Furshpan, and E. Florey. 1953.** Physiological and pharmacological observations on muscle receptor organs of the crayfish, *Cambrus clarkii* Girard. *J. Exp. Biol.* **30:** 136–150.

**Wiersma, C. A. G., and R. L. C. Pilgrim. 1961.** Thoracic stretch receptors in crayfish and rock lobster. *Comp. Biochem. Physiol.* **2:** 51–64.

**Yamagishi, H. 1985.** Spontaneous activity and pacemaker property of neurons in the cardiac ganglion of an isopod, *Ligia exotica*. *Comp. Biochem. Physiol.* **81A:** 55–62.